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OCCURRENCE OF *ARMILLARIA ECTYPA* (Fr.) Lamoure IN PEAT BOGS OF THE AUVERGNE — THE REPRODUCTION SYSTEM OF THE SPECIES

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SUMMARY — *Armillaria ectypa* (Fr.) Lamoure is regarded as the only *Armillaria* species throughout the world which is not linked to trees or shrubs. This rare species is found in the arctic or mountain peat-bogs of Europe.

A survey of the macromycetes of the peat bogs was carried out in the Auvergne region (Central France). *Armillaria ectypa* was discovered in seven sites, it was particularly abundant (over 100 fruitbodies) in one of them.

The morphology of the fruitbodies and mycelium in culture is described. Previous studies suggested for the species a homothallic system of reproduction. This was confirmed by: i) the morphological similarity of the single-spore isolates from one fruiting body, ii) the absence of mating reactions among these isolates when paired and iii) the similarity of these single spores through RAPD analysis. By contrast, RAPD revealed variability among single-spore mycelia isolated from the fruitbody of a tetrapolar species (*Armillaria ostoyae*).

KEY WORDS: *Armillaria*, Basidiomycetes, peat bogs, ecology, RAPD markers, homothallism.

RÉSUMÉ — *Armillaria ectypa* (Fr.) Lamoure est la seule espèce d'armillaire connue qui ne soit pas lignivore et liée aux écosystèmes ligneux. Cette espèce, qui passe pour très rare, est inféodée aux sphaignes, sa présence a été signalée d'une part en Laponie, d'autre part dans les tourbières de montagne de l'Europe tempérée.

Une prospection des Macromycètes des tourbières de la Région Auvergne a conduit à la découverte d'*A. ectypa* sur 7 sites d'Auvergne (5 dans le Puy-de-Dôme, un en Haute-Loire, un dans le nord-est du Cantal). Son abondance était particulièrement grande (plus de 100 carpophores dénombrés) sur le site cantalien.

La présente publication décrit les carpophores de l'espèce sur ses stations auvergnates, ainsi que la morphologie du mycélium en culture.

Des travaux antérieurs suggéraient pour *A.ectypa* un système sexuel homothalle. De fait, les myçéliums monosporiques obtenus à partir d'un même carpophore sont apparus morphologiquement identiques et leur croisement n'a pas permis de les répartir en plusieurs pôles. L'analyse par RAPD d'une série de 10 monospores issues d'un même carpophore a confirmé l'homogénéité génétique de ces mycéliums. *A contrario*, l' analyse RAPD appliquée aux mycéliums monospores d'une armillaire hétérothalle (*A.ostoyae*) a fait apparaître une importante variabilité.

MOTS-CLÉS: *Armillaria*, Basidiomycètes, tourbières, écologie, marquage par RAPD, homothallisme.

INTRODUCTION

Armillaria ectypa (Fr.) Lamoure is a rare European agaric species specifically growing in peat bogs. This species was created by Fries (*Epichrisis Systematis Mycologici* 1836-1838) under the name *Agaricus ectypus*. Quélet (1881) transfered it to genus *Clitocybe* (Fr.) P. Kumm. Bresadola (1928) also described a fungus named *Clitocybe ectypa*, however according to Moreau & Moreau's analysis (1929), Bresadola's fungus was not the same species as that described by Fries. Lamoure (1965) observed the close taxonomical proximity between *Clitocybe ectypa sensu* Quélet and the forest species *Armillaria tabescens* (Scop.: Fr.) Emel, that earlier authors regarded as a *Clitocybe* species (*Clitocybe tabescens* (Scop.: Fr.) Bres.). *Armillaria tabescens* itself is related to the "true" *Armillaria* species (with annulate stipes) which constitute the complex "*A.mellea sensu lato*" (divided into a number of different species after the studies by Romagnesi (1970, 1973) and Korhonen (1978). *Armillaria ectypa, A. tabescens* and "*A. mellea sensu lato*" share three remarkable traits: initiation of highly-differentiated rhizomorphs in pure culture, bioluminescence of aerial mycelium, and the uninucleate state of the mycelial elements (Lamoure, 1965); beyond a doubt, these common features justify Lamoure's proposal to transfer *Clitocybe ectypa* to genus *Armillaria* (Fr.) P. Kumm. However, this species is unique within the genus by virtue of its ecology: in contrast with all the other *Armillaria* species, which are lignicolous and more or less pathogenic to living trees, *A. ectypa* has only been found in peat-bogs, associated with *Sphagnum*.

The species could also be original by its reproduction system. All the forest, European, *Armillaria* species have been shown to be heterothallic and tetrapolar (Hintikka, 1973; Korhonen, 1978; Guillaumin *et al.*, 1991). By contrast, Guillaumin (1973) suggested for *A. ectypa* a homothallic system of sexual reproduction; this suggestion was based on the morphological identity of the single spore cultures from the same fruitbody, the absence of mating reactions in pairings between these cultures, and formation of fertile fruitbodies in the laboratory from some of these strains of single-spore origin.

Armillaria ectypa has been found in two European areas: Lapland and the mountains of Western Europe. In Lapland, it was recently reported by Ohenoja & Väre (1993). In Western Europe, the species was reported from the Vosges (Quélet, 1881), Schwartzwald (Ohenoja, pers.comm.), Jura (Favre, 1939), the French Alps (Favre, 1939; Lamoure, 1965), the Bavarian Alps (Marxmüller, pers. comm.) and from two peat bogs of the Auvergne (Moreau & Moreau, 1929).

MATERIAL AND METHODS

1) Mycological survey of the peat-bogs of the Auvergne

A mycological survey of the mountain peat-bogs of the Auvergne region has been conducted by one of the current authors (René-Jacques Bouteville). This survey concerned three of the four departments of the region (Puy-de-Dôme, Cantal, Haute-Loire), but was particularly intensive in the south-west area of the Department of Puy-de-Dôme (Monts-Dore, Artense and Cézallier). This survey reported a number of macromycetes (Bouteville, 1991), among which *Armillaria ectypa* was found.

2) Fungal material

Fruitbodies of *A. ectypa* were collected on 2 sites:

a) the peat-bog of Limagne (Département: Haute-Loire, commune: Siaugues-Saint-Romain).
b) the peat-bog of La Chambe (Département du Cantal, commune: Montgreleix).

Pure cultures of the fungus were obtained from these fruitbodies: a) from the sterile parts of the basidiome (stipe and context of the pileus), b) from single basidiospore isolation. The method used for single spore isolation has been previously described (Guillaumin & Berthelay, 1981).

As a control in RAPD studies, we also used single spore cultures of a tetrapolar species:*Armillaria ostoyae* (Romagnesi) Herink.: eight single-spore mycelia were isolated from a basidiome obtained in pure culture. The isolate which had fruited (number PC 79-4 in our collection) had been isolated in 1979 from a diseased Maritime pine (*Pinus pinaster*) at Cestas (Département de la Gironde).

3) Matings

The matings between single-spore cultures were carried out in Petri dishes on malt-agar (malt 2%, agar 1.5%). Inoculum for pairing consisted of undifferentiated mycelium cut from the margin of a growing culture. According to our routine procedures, the two plugs were placed side by side at the centre of the dish.

4) RAPD (Random Amplification of Polymorphic DNA)

a) culture of the fungus

The fungal strains were grown in liquid malt (2%) in plastic flasks at 23°C. The mycelium and rhizomorphs were collected after one month and lyophilised.

b) DNA extraction

Total DNA was extracted according to the method described by Mohammed (1994), slightly modified: each lyophilised sample (about 10-20 mg) was put in an Eppendorf tube of 1.5 ml with 500 µl of TES Buffer (100 mM Tris pH 8.0, 10 mM EDTA, 2% SDS). The sample was ground with sterile sand. Then, 5 µl proteinase K were added

(20 µg/ml) and the sample was incubated for 1 h at 55-60°C. Then, 100 µl of CTAB 10%-NaCl were added (CTAB 10g., NaCl 4,1 g., water qsp 100 ml). After an incubation of 10 min at 65° C, ADN was extracted with 700 µl of a mixture chloroform / isoamylic alcohol (24/1)-SEVAG.

After 30 min at -20°C., 10 min centrifugation at 13500 rpm led to separation into two phases , the upper, aqueous phase was transfered to a new Eppendorf tube, DNA was precipitated with 500 µl of isopropanol at -20° C., then concentrated by another centrifugation (20 min at 13500 rpm). The supernatant was removed and the pellet was rinsed twice with 1 ml of ethanol 70% at -20° C, dried and dissolved in 50 µl TE buffer.

The optical density of each sample was measured with a spectrophotometer at wavelength 260 nm. The samples ready for RAPD analysis were prepared by dilution so as to obtain about 60 ng of DNA in each test tube.

c) RAPD test

The amplification reaction was conducted according to Williams *et al.* (1990), with slight modifications, in a volume of 25 µl with: 10 mM Tris-HCl pH 9, 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X100, 0.2% gelatin, 200 µM each of dATP, dTTP, dCTP, dGTP, 0.2 µM of the primer, 0.5 unit of Taq-polymerase (Appligène) and about 60 ng of DNA.

Amplification was carried out in a 9600 Perkin Elmer Cetusthermal cycler as follows: initial denaturation for 5 min at 93°C, then 40 cycles with the three steps: denaturation 1min at 91°C, annealing 1min at 36°C, polymerisation, 2 min at 70°C. The 40 cycles were followed by final extension for 5 min at 70°.

The amplification products were separated by electrophoresis on 1.4% agarose gel in Tris-acetic-acid EDTA (TAE) buffer and detected by staining with ethidium bromide.

Five different primers were tried: three had been selected by Anderson's team at the University of Toronto: R25 (ACTTGAGGCG), R28 (ATGGATCCGC) and UBC31 (CCGGCCTTCC). These three primers had been shown to reveal a high variability among the isolates of *Armillaria spp.* (Smith *et al.*, 1992, Guillaumin *et al.*, 1996). The other two primers were OPD 20 (ACCCGGTCAC) and OPF 01 (ACGGATCCTG), produced by Operon Technologies, California, (kits D and F), and which had provided with good results at Clermont-Ferrand when applied to *Armillaria* isolates of African origin. (Abomo-Ndongo *et al.* 1997).

RESULTS

1) Distribution of *A. ectypa*

The species was found in seven sites of region Auvergne:
Département HAUTE-LOIRE:

* peat-bog of Limagne (commune: Siaugues-Saint-Romain, altitude: 1083 m).

Département PUY-DE-DOME:

* peat-bog of Bourdouze (commune: Besse-et-Saint-Anastaise, alt.: 1210 m).
* " of Chambedaze (commune: Egliseneuve d'Entraigues, alt.: 1180m).
* " of La Godivelle d'en-Bas (commune: La Godivelle, alt.: 1200 m.)

* " of Les Chastelets (commune: La Godivelle, alt.: 1208 m).
* " of Rimat (commune: Chastreix, alt.: 1237 m.)

Département CANTAL:

* peat-bog of la Chambe (commune: Montgreleix, alt.: 1210m.). This small peat-bog (fig.1) has no name on the maps, we named it from the name of the nearest "buron" (cattle shed).

With the exception of Limagne, located in the region Deves, these sites belong to the geographical region Cézallier-Artense, on the borders of Cantal and Puy-de-Dôme. These peat-bogs fill hollows due either to volcanic activity or to overdeepening by glaciers.

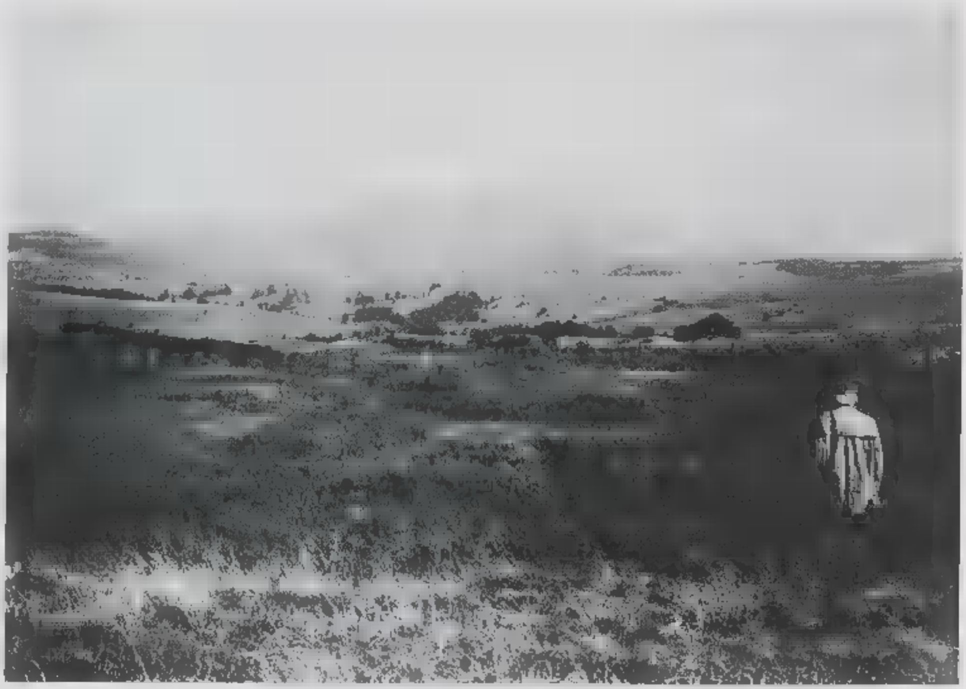

Figure 1 — General aspect of the peat-bog of La Chambe (with "Massif du Sancy" as a background).
Figure 1 — Vue d'ensemble de la toubière de La Chambe (avec le Massif du Sancy en arrière plan).

2) Ecological environment of the species

The number of basidiomes of *A. ectypa* found in 1996 was about 100 at La Chambe, 20 at Limagne, but <10 on the other five sites. The basidiomes of the fungus generally appear in late August. At La Chambe in 1996, the fruiting period extended from 15th August to 8th September.

The base of the vegetation of the bog consists of several species of *Sphagnum* (*S. subsecundum ssp. inundatum, S. platyphyllum, S. flexuosum*) which are partly flooded and constitute a spongy cloth more or less continuous. The angiosperms are typical of sphagnophilous vegetation (*Molinia coerulea, Carex spp., Eriophorum angustifolium., Potentilla palustris, Menyanthes trifoliata, Succisa pratensis, Drosera rotundifolia, Andromeda polifolia*, etc.). The basidiomes of *A. ectypa* appear either disseminated or by groups of 3-4 (fig.2), their stipe is attached to the lower, dead part of the sphagnums through a cottony aggregated mycelium. Rhizomorphs are not found in nature.

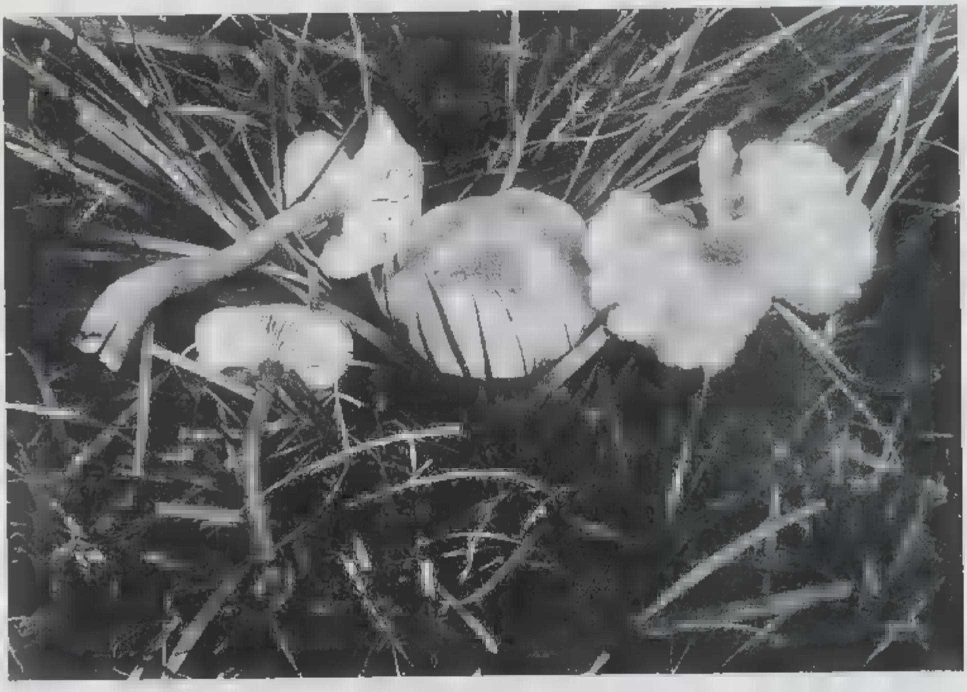

Figure 2 — Fruiting bodies of *A. ectypa in situ* (La Chambe).
Figure 2 — Fructification de *A. ectypa in situ* (La Chambe)

3) Morphology of the species

a)Fruitbodies in natural conditions (fig. 2-3)

The great number of basidiomes found at La Chambe led to observe some variations from the descriptions carried out by Moreau & Moreau (1929) and by Favre (1939).

The young **pileus** is convex, it becomes more flat when growing old, with an incurved or upturned margin. This pileus is frequently hygrophanous or water-soaked. The pileal surface is striate-pellucid, glabrous, glossy. A hand-lens is necessary to detect thin, radiating fibrils which are denser on the central disk. In dry weather, the dense central fibrils can peel off, giving a furfuraceous aspect to this part of the pileus which has often been decribed as glabrous.

The colour of the pileus is russet to brownish, imbued (S202-S203). In dry weather, it becomes paler (beige-ochraceous to sandy) from the centre to the margin, the colour of the central disk remaining darker.

The **lamellae** are scarce, more or less bulging, adnexed to slightly decurrent , in the latter case shortly prolonged on the stipe, more or less bifurcated at the insertion. The edge is smooth, irregular, first whitish then cream, finally brownish, like the margin of the pileus.

The **stipe** is long (10 to 12 cm), but deeply sunken in sphagnum, thin under the pileus (0.5 to 0.8 cm) but wider at the base, hollow. It is fibrillose or silky with brownish fibrils, which protrude over a paler background, soaked and sharing the same colour as the pileus. The stipe is pruinose below the hymenium (2 cm) and cottony at the base.

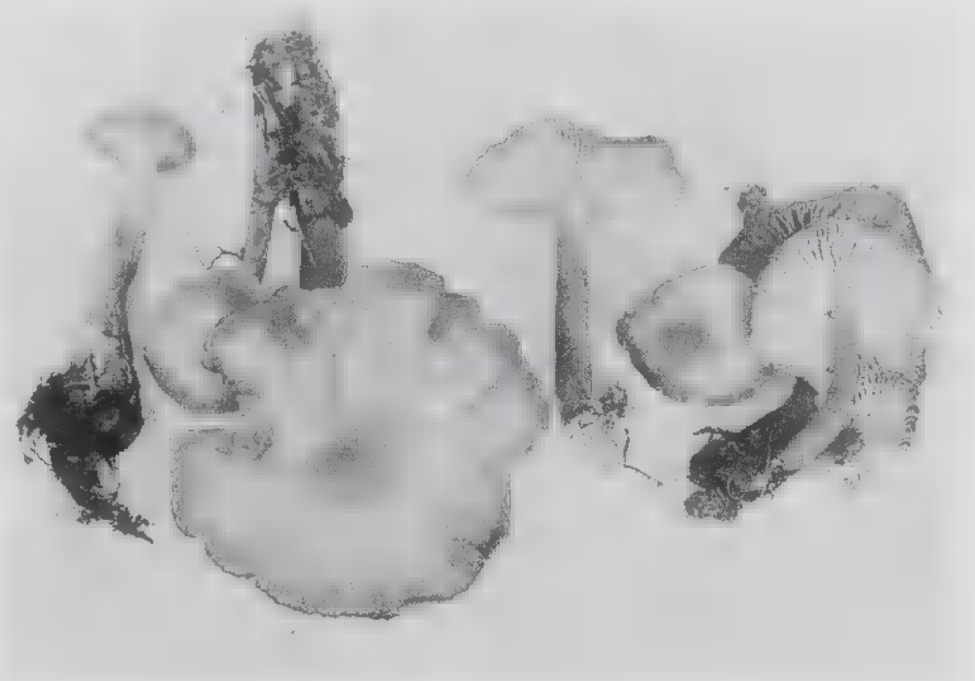

Figure 3 — Fruiting bodies of *A. ectypa* from Limagne
Figure 3 — Fructification de *A. ectypa* de Limagne

The **context** is entirely brownish, however its colour is highly variable according to the wetness of the basidiome. The smell is strong, acidic, recalling both vinegar and anise (as that of the sphagnum). It becomes foetid and unpleasant in dry samples. The taste is mild, slightly astringent after chewing.

The **basidiospores** are smooth, hyaline, ellipsoid, their dimensions are the same as for *A. mellea* or *A. ostoyae* (7-10 × 5-6.5 µm). Spore-print is cream-coloured.

Armillaria ectypa differs from the other exannulate *Armillaria* species *A. tabescens* by the woody habitat of the latter species (generally on oaks or chesnut) , its fasciculate basidiomes, its whitish context and its densely flecked pileus.

Armillaria ectypa fruits easily in the laboratory (fig.4).

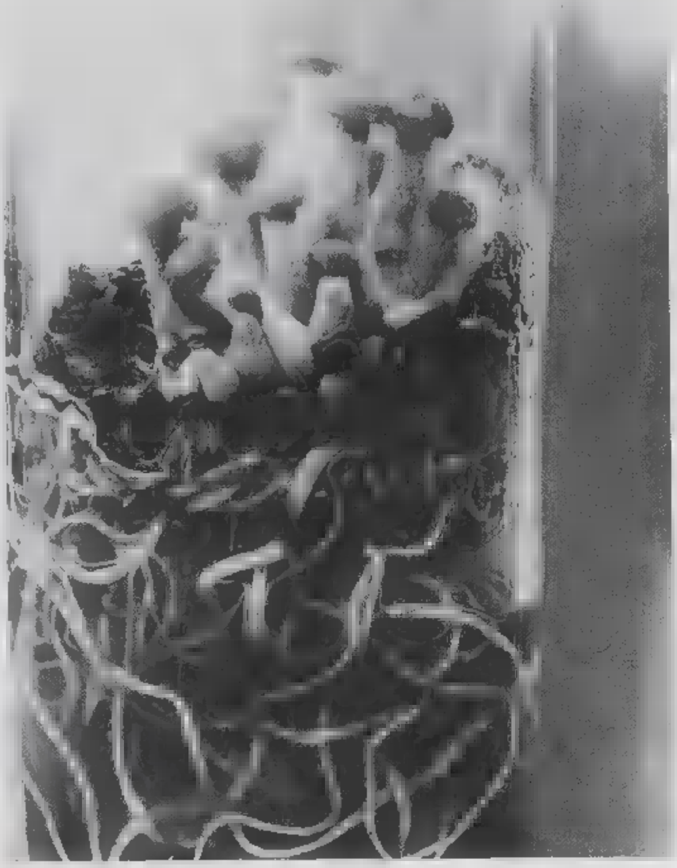

Figure 4 — Fruiting of *A. ectypa* in the laboratory (Isolate PY 72-2 from the French Alps).
Figure 4 — Fructifications de *A. ectypa* obtenues au laboratoire (Isolat PY 72-2 des Alpes françaises).

b) Aspect of the mycelia in pure culture

The morphology of *A. ectypa* in pure culture in standard conditions (culture at 24° in the dark, on malt 2%, agar 1.5%, in Petri dishes 9 cm in diameter) is typical (fig. 5-6). The single spore cultures and the cultures from the context of the basidiome are identical. The fungus grows more quickly than most other *Armillaria* species, it shows an abundant, white aerial mycelium with a strong luminescence. After about 20 days, this mycelium is in places covered by discontinuous brown crusts. The rhizomorphs are abundant, sinuous, moderately branched, strictly cylindrical (diameter: 1-2 mm), they remain white even aged. They are generally intramatricial but can also protrude outside the medium. These rhizomorphs adhere to the bottom of the dish by special short brown, cylindrical, branches (fig. 7).

This morphology is typical of the species. By the white, abundant aerial mycelium and discontinuous crusts, *A. ectypa* resembles *A. mellea* and *A. tabescens*, but these two species have flattened, highly branched, rhizomorphs on malt 2%. On the other hand, *A. gallica* and *A. cepistipes* have, like *A. ectypa*, cylindrical rhizomorphs on malt 2%, but in the first two species, these structures are straight and become dark in old cultures. The adhering rhizomorphic branches also appear to be original structures. Guillaumin *et al.* (1990, unpublished) had included two isolates of *A. ectypa* (from the Auvergne and Bavaria) in a morphological comparison between 58 *Armillaria* isolates. The use of Factorial Analysis of Correspondances had shown that the two isolates of *A. ectypa* appeared very close to each other and far from the other species.

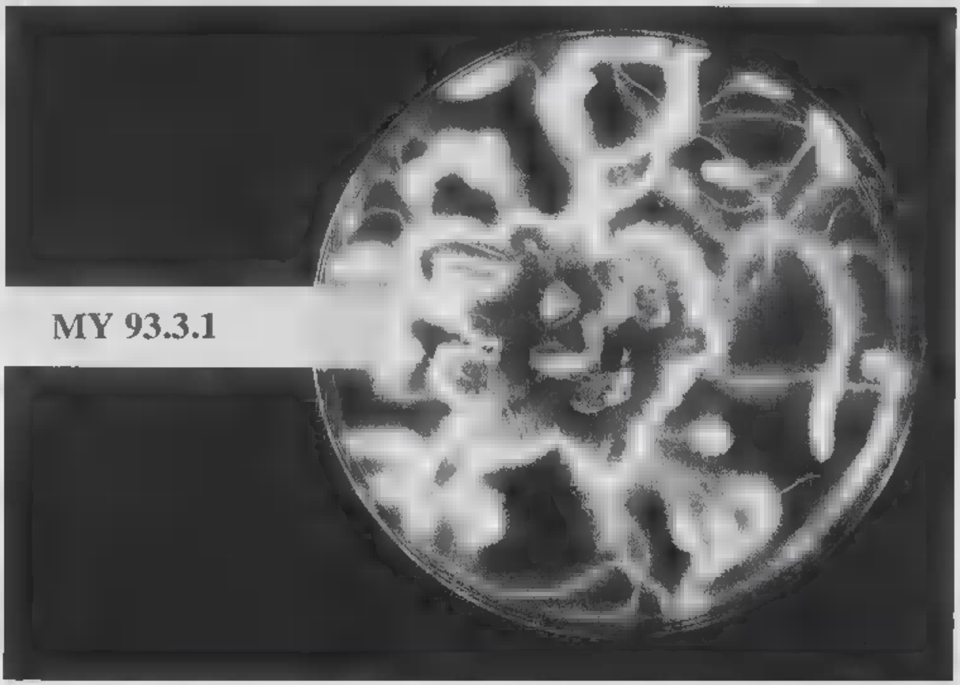

Figure 5 — Mycelium of *A. ectypa* (single-spore isolate MY 93.3.1 from Limagne) aged three weeks: upper side of the Petri dish

Figure 5 — Mycélium de *A. ectypa* (culture monosporale de l'isolat MY 93.31 de Limagne) agé de trois semaines : face supérieure de la boite de Petri.

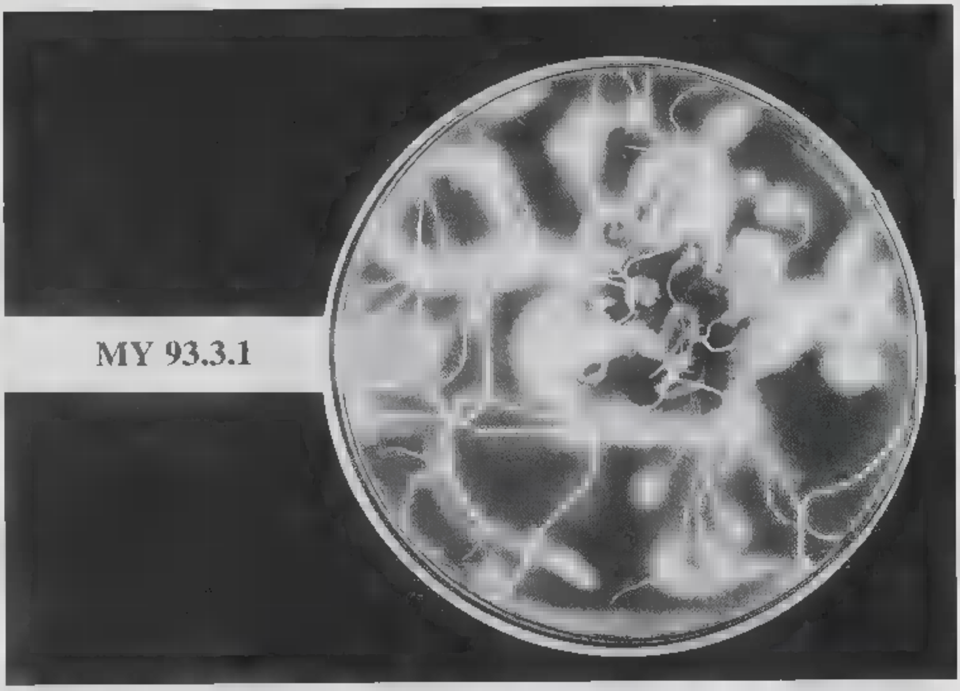

Figure 6 — id., lower side of the Petri dish.
Figure 6 — id., face inférieure de la boite de Petri.

3) Matings

a) 8 single-spore mycelia from a basidiome from Limagne were paired with each other (total: C8/2 = 28 pairings).
b) 10 single-spore mycelia from a basidiome from the peatbog of La Chambe were also paired with each other (total: C10/2 = 45 pairings).
c) 3 single-spore cultures from Limagne were then paired with 3 single-spore cultures from La Chambe (9 pairings).

No mating behaviour could by observed from any of these 82 pairings. The different strains, which were morphologically very similar (even from the two different sites) seemed to fuse with each other without any morphological modification of the mycelia or incompatibility reactions. After 1 month, in most cases it was impossible to distinguish between the thalli of the two paired strains.

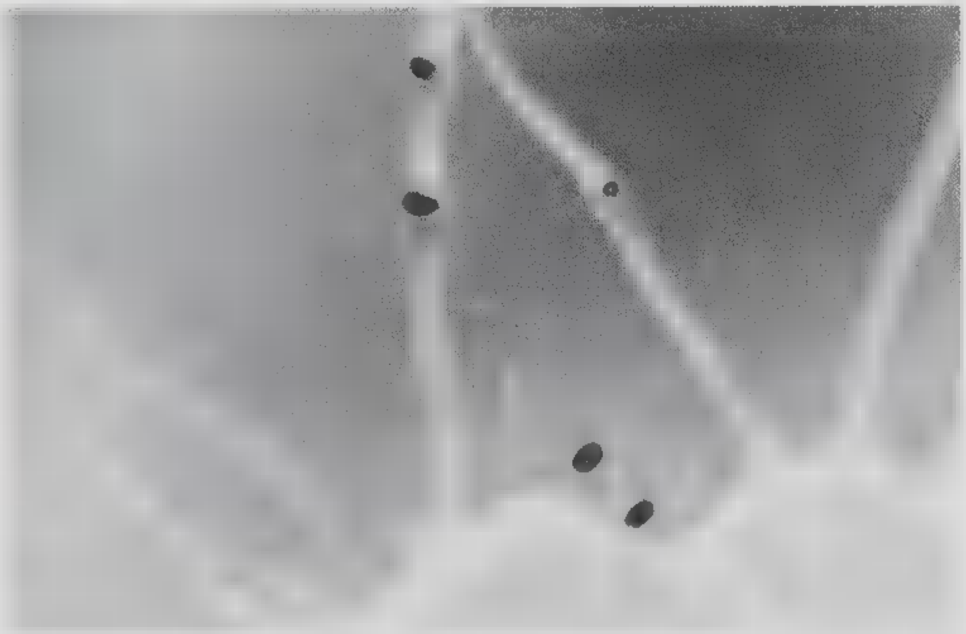

Figure 7 — Rhizomorphs in culture with adhesive branches.
Figure 7 — Rhizomorphes en culture avec ramifications adhésives.

4) RAPD

* In a first step, 10 single spore cultures of *A. ectypa* from La Chambe were investigated with primer UBC31 in comparaison with 8 single-spores of *A. ostoyae* originating from a fruitbody of the isolate PC 79-4.

The ten single-spore cultures of *A. ectypa* from the same fruitbody appeared completely identical, all exhibiting 9 bands at the same rF. By contrast, an important variability appeared among the single-spore cultures of *A. ostoyae*: 11 potential major bands were found for the eight strains as a whole, of which 4 were common to all 8 strains. From the other 7 major bands, only two strains appeared similar (n° 3 and 8), all the others were different from each other:

PC 79-4-1 : bands 1, 3, 6,7,8,9,10,11
PC 79-4-3 : bands 3,4,5,6,7, 9,10,11
PC 79-4-4 : bands 2,3,4, 6,7, 9,10,11
PC 79-4-5 : bands 2,3, 6,7,8,9,10,11
PC 79-4-6 : bands 4,5,6,7, 10,11
PC 79-4-8 : bands 3,4,5,6,7, 9,10,11
PC 79-4-9 : bands 2, 5,6,7, 10,11
PC 79-4-10 : bands 3,4,5,6,7,8,9,10,11

* In a second step, only five single spores of *A. ectypa* were analysed, with, as a heterothallic control, only four single spores of *A. ostoyae* (numbers PC 79-4-3, 5, 8 and 9). Four different primers were tried: R25, R28, OPD20 and OPF01.

— with R25 (fig.8), the five single-spores of *A. ectypa* were identical, with five major and five minor bands. The four single-spores of *A. ostoyae,* had, as a whole, 9 potential major bands, and numbers 5 and 8 were identical:

PC 79-4-3 : bands 1,2, 4, 6,7,8,9
PC 79-4-5 and 8 : bands 2,3,4, 6,7,8,9
PC 79-4-9 : bands 1,2, 4,5, 7,8,9

— with R28, the five single-spores of *A. ectypa* were identical, all of them showing 8 major bands at the same rF. The four single-spores of *A. ostoyae* had, as a whole, 7 major bands and were easily differentiated from each other:

PC 79-4-3 : bands 2, 5, 7,
PC 79-4-5 : bands 2, 5,6,7
PC 79-4-8 : bands 2, 4,5
PC 79-4-9 : bands 1,2,3, 5,6

— with OPD20 (fig.8), the five single-spores of *A. ectypa* were identical, with only 1 major band and some 10 minor bands. The four single-spores of *A. ostoyae* had only five major bands, and could however be differentiated from each other:

PC 79-4-3 : bands 1, 3, 5
PC 79-4-5 : bands 1, 3,4,5
PC 79-4-8 : bands 1,2,3,4,5
PC 79-4-9 : bands 1,2,3, 5

— with OPF01, the five single-spores of *A. ectypa* were identical, all of them showing 1 major and 5 minor bands at the same rF. As concerned *A. ostoyae*, the DNA of the mycelium of PC 79-4-5 had not been amplified. The numbers 3 and 9 appeared similar (3 major bands at the same rF) and the number [illegible] was different, with one of the three bands lacking.

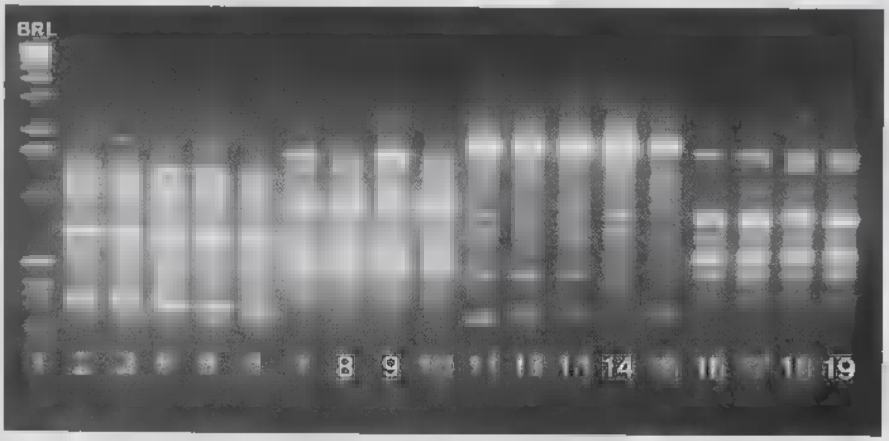

Figure 8 — RAPD analysis of single spores of *A. ectypa* and *A. ostoyae* : lanes 2-6: *A. ectypa*, primer R25; lanes 7-10: *A. ostoyae*, primer R25; lanes 11-15: *A. ectypa*, primer OPD 20; lanes 16-19: *A. ostoyae*, primer OPD 20.

Figure 8 — Analyse en RAPD de souches monosporiques de *A. ectypa* et *A. ostoyae*: 2-6: *A. ectypa*, primer R25; 7-10: *A. ostoyae*, primer R25; 11-15: *A. ectypa*, primer OPD 20; 16-19: *A. ostoyae*, primer OPD 20.

DISCUSSION AND CONCLUSION

Armillaria ectypa seems to be a strictly European, arctico-alpine species. Perhaps a relict of the ice age, it is confined to *Sphagnum* peatbogs of high latitude or altitude. Even in Lapland, it is not a common species and seems to be declining (Ohenoja & Väre, 1993). It is quite rare in the mountains of temperate Europe where, to our knowledge, it has not still been reported from massifs such as the Pyrénées or the Carpathians. However, our study shows that it is particularly frequent in the Cézallier, a volcanic plateau of the Massif Central at altitude 1100-1300 m., which has been restructured by quaternary glaciations. The Cézallier harbours several angiosperms which are relicts from the ice age, for instance the very rare Asteraceae *Ligularia sibirica*, which is abundant at Les Chastelets where it coexists with *A. ectypa*.

In contrast to several forest *Armillaria* species, highly pathogenic for trees, *A. ectypa* has no economic importance. However, this species is interesting from two points of view: its phylogenetic relationships with the forest *Armillaria* species and its reproduction system.

A recent study based on ITS sequencing (Chillali *et al.*, 1997), shows that *A. ectypa* is genetically more distant from all the other European *Armillaria* species (including *A. tabescens*) than these species are from one another. In 1979, Lung-Escarmant and Dunez, using immunoenzymatic methods, had drawn similar conclusions. It is likely that a common ancestor of all the forest *Armillaria* species (maybe close to *A. tabescens*) has early diverged from a peat-bog species. The former would have given rise to a number of wood-colonizing saprophytic or parasitic species while the latter, confined in the particular environment of the peat-bogs, would have remained more or less stable.

The caryological cycle and reproduction system are other original traits of the species. *Armillaria ectypa* is certainly homothallic. The fruiting capacities of single-spore isolates (Guillaumin, 1973), absence of mating reactions, morphological identity between single-spore mycelia and isolates from the context of the basidiome, were good arguments which are strengthened by the demonstration, given by the present study, of genetical identity of the single-spore isolates from the same basidiome. The basidia have regularly four spores, the basidiospores are uninucleate, a normal meiosis seems to occur in the basidium (Guillaumin, unpublished). Therefore, the hypothesis of primary homothallism can be proposed in preference to secondary homothallism or apomixy. The mycelium would be diploid, as in the other *Armillaria* species, the basidiospores would be haploid, and a self-diploidization would take place in the early stages of the growth of the mycelium, maybe during germination of the basidiospore. However, this self-diploidization remains completely hypothetical and needs confirmation by cytological observations of young germinations.

Recent studies have revealed that other *Armillaria* species have a homothallic reproduction system:*A. mellea ssp. africana, A. mellea ssp. nipponica*, the Caribbean species *A. puiggarii* Speg. and certain isolates of the African species *A. heimii* Pegler (Mohammed & Guillaumin, 1993; Cha & Igarashi, 1995; Abomo-Ndongo *et al.*, 1997). Except *A. mellea ssp. nipponica*, these species are tropical. Like *A. ectypa*, they are suspected of having a diploid cycle and an early self-diploidization in the mycelium. However, these taxa differ from *A. ectypa* by the absence of dikaryons and clamps in the hymenium. By contrast, *A. ectypa* is regularly clamped in the subhymenium (Lamoure, 1965; Guillaumin, 1973). By this characteristic, it resembles some heterothallic species like *A. ostoyae* (Romagn.) Herink, *A. gallica* Marxmüller & Romagn. or *A. borealis* Marxmüller & Korhonen. On the other hand, the tropical homothallic species are similar to an other group of temperate, heterothallic species including *A. mellea ssp. mellea* and all the Australian *Armillaria* species, which all lack clamp connexions in the hymenium. The origin of these transitory clamped elements is not really understood, even in the context of heterothallism; they suppose a vegetative haploidization in the young basidiome.

An additional characteristic of *A. ectypa* is the absence, in this species, of the phenomenon of intraspecific Somatic Incompatibility (SI): the pairing of isolates from different geographical origins results in merging of mycelia without any antagonistic reaction. *A. ectypa* shares this behaviour with the African, partly homothallic species *A. heimii* (Abomo-Ndongo Guillaumin, 1997) but differs from all the tetrapolar, temperate *Armillaria* species in which intraspecific SI is a general and very spectacular phenomenon (Guillaumin *et al.*, 1991). The biological consequence of SI in these tetrapolar species is the vegetative isolation of the different genotypes or "genets" (Legrand *et al.*, 1996), which can exchange genes through sexual reproduction, but not through vegetative anastomoses; thus, heterokaryosis is strongly limited in these species. The absence of SI in *A. ectypa* could have two explanations: (i) either the species consists of one and the same clone at least in all the European mountains (the pairing with arctic isolates has not been attempted yet), (ii) or the different genotypes can exchange nuclei without any obstacle, leading to general heterokaryosis within the species. The analysis of variability between isolates of different geographical origins (for instance with the tools of RAPD) should allow a choice between the two hypotheses.

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